Supplemental Information

Efficient Non-viral Gene Delivery into Human Hematopoietic Stem Cells by Minicircle Sleeping Beauty Transposon Vectors

Marta Holstein, Cristina Mesa-Nuñez, Csaba Miskey, Elena Almarza, Valentina Poletti, Marco Schmeer, Esther Grueso, Juan Carlos Ordóñez Flores, Dennis Kobelt, Wolfgang Walther, Manish K. Aneja, Johannes Geiger, Halvard B. Bonig, Zsuzsanna Izsvák, Martin Schleef, Carsten Rudolph, Fulvio Mavilio, Juan A. Bueren, Guillermo Guenechea, and Zoltán Ivics
Supplementary Figure S1. Minicircle Sleeping Beauty transposon vectors. a) Agarose gel electrophoresis of the minicircle MC.T2-CAGGS-Venus (MC1390). Lanes M: 1-kb DNA size marker (PlasmidFactory Item no. MSM-865-50), lanes 1,2: undigested minicircle DNA almost monomeric supercoiled form, lanes 3,4: minicircle DNA linearized with Pmel (NEB) to determine the size (appr. 3.4 kb). b) Agarose gel electrophoresis of the minicircle MC.SB100X (MC1420). Lanes M: 1-kb DNA size marker (PlasmidFactory Item no. MSM-865-50), lanes 1,2: undigested minicircle DNA almost monomeric supercoiled form, lanes 3,4: minicircle DNA linearized with PacI (NEB) to determine the size (appr. 2.3 kb).
Supplementary Figure S2. Long-term persistence of transgene expression in CD34+ cells following Sleeping Beauty transposon-mediated stable gene delivery in vitro. Percentages of Venus-expressing cells after nucleofection of CD34+ cells with (MC-SB 10+5) or without (MC-SB 10+0) SB transposase.
Supplementary Figure S3. Comparative numbers of colonies generated after nucleofection of CD34+ cells determined by CFU assays. Numbers of total CFUs after seeding of 4,000-4,500 purified CD34+ cells following nucleofection with MC DNA only and with MC DNA/SNIM.RNA are shown. Data are expressed as means ± SEM; n = 3 per group.
Supplementary Figure S4. Sequence logo of Sleeping Beauty insertion sites in CD34+ cells. The figure was generated on the base frequency matrix of genomic sequences immediately flanking the SB insertion sites in HCSs using the seqLogo package in R.
Supplementary Figure S5. Distribution of Sleeping Beauty transposon, MLV-derived gammaretroviral and HIV-1-derived lentiviral integrants in a 25-state chromatin segmentation dataset of CD34⁺ cells. Color intensities depict the degree of deviation from the expected random distribution. The cladogram was drawn on the basis of row means. The abbreviations stand for the following genomic states:

- **TssA**: Active TSS
- **PromU**: Promoter Upstream TSS
- **PromD1**: Promoter Downstream TSS 1
- **PromD2**: Promoter Downstream TSS 2
- **Tx5**: Transcribed - 5' preferential
- **Tx**: Strong transcription
- **Tx3**: Transcribed - 3' preferential
- **TxW**: Weak transcription
- **TxReg**: Transcribed & regulatory (Prom/Enh)
- **TxEnh5**: Transcribed 5' preferential and Enh
- **TxEnh3**: Transcribed 3' preferential and Enh
- **TxEnhW**: Transcribed and Weak Enhancer
- **EnhA1**: Active Enhancer 1
- **EnhA2**: Active Enhancer 2
- **EnhAF**: Active Enhancer Flank
- **EnhW1**: Weak Enhancer 1
- **EnhW2**: Weak Enhancer 2
- **EnhAc**: Primary H3K27ac possible Enhancer
- **DNase**: Primary DNase
- **ZNF/Rpts**: ZNF genes & repeats
- **Het**: Heterochromatin
- **PromP**: Poised Promoter
- **PromBiv**: Bivalent Promoter
- **ReprPC**: Repressed Polycomb
- **Quies**: Quiescent/Low
Supplementary Figure S6. Viral gene vector insertions have a tendency to associate with genes also linked to human disease. Shown are the statistically most significant human phenotype ontology categories in association with vector insertions within expressed genes of human HSCs. The GREAT algorithm\(^1\) was used to calculate association based on two measures of enrichment: a binomial test over genomic regions and a hypergeometric test over genes. Any target region was considered statistically significant if the FDR Q-values for both statistical tests were $\leq 0.05$. Asterisks mark phenotypes related to hematopoietic functions.

Supplementary Figure S7: Representative analyses showing the expression of the Venus marker gene in human myeloid (CD33), lymphoid (CD19) and CD34+ cells engrafting NSG mice. Data correspond to one mouse transplanted with cells nucleofected with the MC/SNIM.RNA-SB constructs.